DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002083872	A2	20021024	WO 2002-US12405	20020417

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-837306 A 20010417
US 2001-516 A 20011024
US 2001-45674 A1 20011025

AB The invention concerns methods useful in constructing libraries that collectively display and/or express members of diverse families of peptides, polypeptides or proteins and the libraries produced using those methods. Methods of screening those libraries and the peptides, polypeptides or proteins identified by such screens.

L19 ANSWER 2 OF 51 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2002:244158 BIOSIS
DOCUMENT NUMBER: PREV200200244158
TITLE: Intrabodies: Targeting ***scFv*** expression to eukaryotic intracellular compartments
AUTHOR(S): Cohen, Pascale A. (1)
CORPORATE SOURCE: (1) Faculte de Pharmacie, Universite Montpellier I, Montpellier France
SOURCE: O'Brien, Philippa M. [Editor]; Aitken, Robert [Editor]. Methods in Molecular Biology, (2002) No. 178, pp. 367-378. Methods in Molecular Biology. Antibody phage display: Methods and protocols. print.
Publisher: Humana Press Inc. 999 Riverview Drive, Suite 208, Totowa, NJ, 07512, USA.
ISSN: 0097-0816. ISBN: 0-89603-711-8 (paper).
DOCUMENT TYPE: Book
LANGUAGE: English

[redacted]
libraries
AUTHOR(S): Lennard, Simon (1)
CORPORATE SOURCE: (1) Cambridge Antibody Technology, The Science Park, [redacted]

SOURCE: O'Brien, Philippa M. [Editor]; Aitken, Robert [Editor]. Methods in Molecular Biology, (2002) No. 178, pp. 59-71. Methods in Molecular Biology. Antibody phage display: Methods and protocols. print.
Publisher: Humana Press Inc. 999 Riverview Drive, Suite 208, Totowa, NJ, 07512, USA.
ISSN: 0097-0816. ISBN: 0-89603-711-8 (paper).
DOCUMENT TYPE: Book
LANGUAGE: English

L19 ANSWER 4 OF 51 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2002239284 MEDLINE
DOCUMENT NUMBER: 21838018 PubMed ID: 11849927
TITLE: A generic strategy for subcloning antibody variable regions from the ***scFv*** ***phage*** display vector pCANTAB 5 E into pASK85 permits the economical production of F(ab) fragments and leads to improved recombinant immunoglobulin stability.
AUTHOR: Kramer Karl; Fiedler Markus; Skerra Arne; Hock Bertold
CORPORATE SOURCE: Department fur Pflanzenwissenschaften, Lehrstuhl fur Botanik, Wissenschaftszentrum Weihenstephan fur Ernahrung, Landnutzung und Umwelt, Technische Universitat Munchen, Alte Akademie 12, D-85350, Freising-Weihenstephan, Germany.. kramer(a)wzw.tum.de
SOURCE: BIOSENSORS AND BIOELECTRONICS, (2002 Apr) 17 (4) 305-13.
Journal code: 9001289. ISSN: 0956-5663.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200210
ENTRY DATE: Entered STN: 20020430
Last Updated on STN: 20021010
Entered Medline: 20021008

AB Apart from the decisive sensitivity and specificity of immunosensors, the employed antibodies essentially contribute to additional key factors like fabrication costs for sensor chips and sensor stability. A production scheme for recombinant antibody fragments has been optimised with respect to these particular issues of biosensor development. The phagemid vector pCANTAB 5 E is widely used for the selection of antibody fragments from corresponding libraries. However, large-scale production of the selected single-chain F(v) (***scFv***) fragments is substantially ***restricted*** by the high cost for the inducer IPTG and the anti-E-tag antibody. The latter is needed in significant amounts for the purification of the recombinant protein. A generic strategy was

[illegible] anti-s-triazine antibody K47H served as a model system in this study. Biosynthesis of the F(ab) fragment in a high cell density fermenter was induced by addition of anhydrotetracycline. The F(ab) fragment was [illegible]

immobilized metal affinity chromatography (IMAC). A yield of 100 microg lxOD(550) purified F(ab) fragment was obtained employing a standard fermentation scheme. The sensitivity and cross-reactivity of the F(ab) was comparable to the parent ***scFv*** when assayed by enzyme immunoassay. However, the F(ab) fragment exhibited significantly improved long-term stability.

L19 ANSWER 5 OF 51 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER 2002:98272 CAPLUS
DOCUMENT NUMBER: 137:61618
TITLE: Chain shuffling to modify properties of recombinant immunoglobulins
AUTHOR(S): Lantto, Johan; Jirholt, Pernilla; Barrios, Yvelise; Ohlin, Mats
CORPORATE SOURCE: Department of Immunotechnology, Lund University, Lund, Swed.
SOURCE: Methods in Molecular Biology (Totowa, NJ, United States) (2002), 178(Antibody Phage Display), 303-316
CODEN: MMBIED; ISSN: 1064-3745
PUBLISHER: Humana Press Inc.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review describes a method for recombining an antigen-specific heavy-chain (HC) and/or light-chain (LC) sequence with sequence variants of the other chain by chain shuffling. The method is based on overlapping polymerase chain reaction between two fragments encoding the HCs and LCs of the antibody, followed by insertion of the complete ***scFV*** gene into a ***phage*** -display vector by conventional enzymic ***restrictions*** and ligation. The benefits of this technique are that the entire cloning procedure requires only two rare-cutting ***restriction*** endonucleases and that it can easily be utilized for other shuffling approaches, such as complementary detg. region shuffling.
REFERENCE COUNT: 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L19 ANSWER 6 OF 51 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER 2002:130763 CAPLUS
DOCUMENT NUMBER: 137:92250
TITLE: ***Phage*** antibody fragments library combining a single human light chain variable region with immune mouse heavy chain variable regions
AUTHOR(S): Rojas, Gertrudis; Almagro, Juan Carlos; Acevedo, Boris; Gavilondo, Jorge V.
CORPORATE SOURCE: Recombinant Antibodies Laboratory, Pharmaceuticals Division, Center for Genetic Engineering and

DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors describe the construction of a ***phage*** antibody

human light chain variable region (VL) with a diverse set of heavy chain variable regions, from a mouse immunized with the prostate specific antigen (PSA). Despite VL ***restriction*** , selection from this library rendered two different single chain Fv antibody fragments, specifically recognizing PSA. The human VL, used as a general partner for mouse heavy chains, was constructed by linking the germline A27 gene and the JK1 minigene segment, both of which are prominently involved in human antibody responses. Our approach offers a fast and simple way to produce half-human mols., while keeping the advantage of immunizing animals for high affinity antibodies.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L19 ANSWER 7 OF 51 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2

ACCESSION NUMBER: 2002:347012 BIOSIS

DOCUMENT NUMBER: PREV200200347012

TITLE: Single-chain Fv antibody-alkaline phosphatase fusion proteins produced by one-step cloning as rapid detection tools for ELISA.

AUTHOR(S): Rau, Doris (1); Kramer, Karl; Hock, Bertold

CORPORATE SOURCE: (1) Center of Life Sciences Weihenstephan, Department of Plant Sciences, Technische Universitaet Muenchen, Alte Akademie 12, D-85350, Freising-Weihenstephan: rau@weihenstephan.de Germany

SOURCE: Journal of Immunoassay & Immunochemistry, (May, 2002) Vol. 23, No. 2, pp. 129-143. http://www.dekker.com/servlet/product/productid/IAS. print.
ISSN: 1532-1819.

DOCUMENT TYPE: Article

LANGUAGE: English

AB A system was constructed for the production of alkaline phosphatase (aP)-labeled antibody single-chain Fv (***scFv***) fragments in Escherichia coli. The expression vector pASK75 was modified by sequentially inserting the E. coli aP coding region and the ***scFv*** cloning cassette. Engineering the cloning sites SfiI and NotI located at the 5' and 3' end of the ***scFv*** gene provides an easy means to insert ***scFv*** fragments. These cloning sites are widely used in recombinant antibody technology and, thus, enable the one-step cloning of ***scFv*** fragments derived from corresponding antibody ***phage*** libraries into the expression vector. An expressed herbicide-specific ***scFv*** -aP fusion protein retained both, analyte binding and enzymatic activity, as determined by ELISA. Therefore, this system permits the production of ***scFv*** -aP conjugates in E. coli, which can replace conventionally prepared aP-labeled antibodies in immunoassays

L19 ANSWER 8 OF 51 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V. DUPLICATE 3

ACCESSION NUMBER: 2002231444 EMBASE

TITLE: Isolation of endotoxin-specific antibodies by selection of

AUTHOR: Chen M.; Yu L.-L.; Zhang X.; Fu W.-L.
CORPORATE SOURCE: W.-L. Fu, Department of Laboratory Diagnosis, South-western Hospital, Third Military Medical University, Chongqing 400038, China. weilingfu@yahoo.com
SOURCE: Chinese Journal of Cancer Research, (2002) 14 2 (118-121).
Refs: 18
ISSN: 1000-9604 CODEN: CJCRFH
COUNTRY: China
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
026 Immunology, Serology and Transplantation
027 Biophysics, Bioengineering and Medical Instrumentation
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Objective: To isolate murine anti endotoxin single chain ***phage*** antibody from a constructed library. Methods: Total RNA was firstly extracted from murine splenic cells and mRNA was reverse-transcribed into cDNA. Then the designed primers were used to amplify the variable region genes of the heavy and light chain (VH, VL) with polymerase chain reaction. The linker was used to assemble the VH and VL into ***ScFv*** , and the NotI and SfiI ***restriction*** enzymes were used to digest the ***ScFv*** in order to ligate into the pCANTAB5E phagemid vector that was already digested with the same ***restriction*** enzymes. The ligated vector was then introduced into competent E.coli TG1 cells to construct a single-chain ***phage*** antibody library. After rescued with M13KO7 helper ***phage*** , recombinant phages displaying ***ScFv*** fragments were harvested from the supernatant and selected with endotoxin. The enriched positive clones were reinfected into TG1 cells. Finally, 190 clones were randomly selected to detect the anti endotoxin antibody with indirect ELISA. Results: The titer of anti endotoxin in murine sera was 1:12,800. The concentration of total RNA was 12.38 .mu.g/ml. 1.9x10(7) clones were obtained after transformed into TG1. 3x10(4) colonies were gotten after one round panning. Two positive colonies were confirmed with indirect ELISA among 190 randomly selected colonies. Conclusion: A 1.9x10(7) murine anti endotoxin single chain ***phage*** antibody library was successfully constructed. Two anti endotoxin antibodies were obtained from the library.

L19 ANSWER 9 OF 51 MEDLINE
ACCESSION NUMBER: 2002103269 MEDLINE
DOCUMENT NUMBER: 21822757 PubMed ID: 11833081
TITLE: Expression and bioactivity identification of soluble MG7 ***scFv*** .
AUTHOR: Yu Zhao-Cai; Ding Jie; Pan Bo-Rong; Fan Dai-Ming; Zhang

SOURCE: World J Gastroenterol, (2002 Feb) 8 (1) 99-102
Journal code: 100883448. ISSN: 1007-9327.
PUB. COUNTRY: China

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020209
Last Updated on STN: 20020505
Entered Medline: 20020503

AB AIM: To examine the molecular mass and identify the bioactivity of MG7 ***scFv*** for its application as a targeting mediator in gene therapy of gastric cancer. METHODS: Two strongly positive recombinant ***phage*** clones screened from MG7 recombinant ***phage*** antibody library were separately transfected into E.coli TG1. Plasmid was isolated from the transfected E.coli TG1 and digested by EcoR I and Hind III to examine the length of exogenous ***scFv*** gene. Then, the positive recombinant ***phage*** clones were individually transfected into E.coli HB2151.The transfectant was cultured and induced by IPTG. Perplasmic extracts was prepared from the induced transfectant by osmotic shock. ELISA was used to examine the antigen-binding affinity of the soluble MG7 ***scFv*** . Immunodotting assay was adopted to evaluate the yield of soluble MG7 ***scFv*** produced by transfected E.coli HB2151. Western blot was used to examine the molecular mass of MG7 ***scFv*** . Finally, the nucleotide sequence of MG7 ***scFv*** was examined by DNA sequencing. RESULTS: Two positive recombinant ***phage*** clones were found to contain the exogenous ***scFv*** gene. ELISA showed that MG7 ***scFv*** had strong antigen-binding affinity. Immuodotting assay showed that transfected E.coli HB2151 could successfully produce the soluble MG7 ***scFv*** with high yield via induction by IPTG. The molecular mass of MG7 ***scFv*** was 30 kDa by western blot. DNA sequencing demonstrated that the VH and VL genes of MG7 ***scFv*** were 363 bp and 321 bp,respectively. CONCLUSION: We have successfully developed the soluble MG7 ***scFv*** which possessed strong antigen-binding affinity.

L19 ANSWER 10 OF 51 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 2002430318 MEDLINE
DOCUMENT NUMBER: 22174580 PubMed ID: 12186782
TITLE: ***Phage*** display of recombinant antibodies toward Burkholderia pseudomallei exotoxin.
AUTHOR: Nathan Sheila; Li Hongbin; Mohamed Rahmah; Embi Noor
CORPORATE SOURCE: Faculty of Science and Technology, Centre for Gene Analysis and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor D. E., Malaysia.. sheila@pkrisc.cc.ukm.my
SOURCE: J Biochem Mol Biol Biophys, (2002 Feb) 6 (1) 45-53.
Journal code: 9714994. ISSN: 1025-8140.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

[illegible]
Last Updated on STN: 20021010
Entered Medline: 20021009

AB We have used the phagemid pComb3H to construct recombinant phages [illegible]

exotoxin of Burkholderia pseudomallei. Variable heavy and light chain fragments were amplified from the hybridoma 6E6A8F3B line, with a wide spectrum of primers specific to mouse antibody genes. Through overlapping extension polymerase chain reaction, the heavy and light chain fragments were linked to form the ***ScFv*** which was subsequently cloned into the ***phage*** display vector and transformed into ER2537 cells to yield a complexity of 10(8) clones. The transformants were screened by four rounds of biopanning against the exotoxin and resulted in selective enrichment of exotoxin-binding antibodies by 301 fold. The ***phage*** pool from the final round of selection displayed antibodies of high-affinity to the exotoxin as demonstrated by ELISA. Several clones were selected randomly from this pool and analysed by ***restriction*** enzyme digestion, fingerprinting and sequencing. ***Restriction*** analysis confirmed that all clones carried a 700-800 bp insert whose sequences, in general, corresponded to that of mouse IgG. Fingerprinting profiles delineated the antibodies into two families with different CDR sequences.

L19 ANSWER 11 OF 51 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:645236 CAPLUS
DOCUMENT NUMBER: 135:225861
TITLE: Chicken type monoclonal antibody
INVENTOR(S): Matsuda, Haruo; Nakamura, Naoto
PATENT ASSIGNEE(S): Foundation for Scientific Technology Promotion, Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 25 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2001238676	A2	20010904	JP 2000-54875	20000229

AB Provided is a recombinant DNA method for quant. producing chicken type monoclonal antibodies. Also, provided are novel plasmid vectors and novel primers for prodn. of the chicken type monoclonal antibodies. The method is a ***phage*** display method comprising insertion of genetic sequence encoding chicken C lambda. chain (i.e. light chain const. region) and gene sequence encoding chicken type monoclonal antibody ***scFv*** into novel expression vector pCPDS and amplification of the expression vector by novel primer CLF1.

L19 ANSWER 12 OF 51 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:719717 CAPLUS

[redacted]

Han, Hua; Niu, Liguo
CORPORATE SOURCE: Department of Cardiology, First Hospital of Xi'an Jiaotong University, Xi'an, 710061, Peop. Rep. China

[redacted]

CODEN: DJDXEG; ISSN: 1000-2790
PUBLISHER: Disi Junyi Daxue Xuebao Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB The ouabain ***ScFv*** (single chain fragment variable) antibody library was constructed by ***phage*** display. Mice were immunized by OUA-OVA (ouabain ovalbumin). MRNA was isolated from the spleen lymphocyte and reversely transcripted. The heavy-chain and kappa light chain variable region genes repertoirs of immunoglobin were amplified individually and assembled into ***ScFv*** by a linker with overlap extension PCR. ***ScFv*** was reamplified with RS primer, meantime SfiI and NotI ***restriction*** sites were added to the ***ScFv*** different ends. ***ScFv*** was digested by SfiI and NotI, and cloned into the phagemid pCANTAB 5E and introduced into E.coli TG1 by electroporation. Phagemid-contg. bacterial colonies were infected with M13KO7 and the ***ScFv*** fusion protein was displayed on the surface of recombinant ***phage*** . Titer of rat serum was 1.2 x 106 after being immunized with OUA-OVA. The VH, VL fragment was 340 bp and 325 bp, resp. After assembling, the ***ScFv*** was about 720 bp. The library capacity was 1.2 x 108. 10 Clones of 12 clones contained 2.1 kb fragment after EcoRI and Hind III digestion. Transfection test indicated that the titer of the culture supernatant was about 9x1014 pfu L-1. A ***phage*** display library of ouabain ***ScFv*** antibody was constructed successfully.

L19 ANSWER 13 OF 51 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 2002149875 MEDLINE
DOCUMENT NUMBER: 21930837 PubMed ID: 11819819
TITLE: Preparation of single chain variable fragment of MG(7) mAb by ***phage*** display technology.
AUTHOR: Yu Z C; Ding J; Nie Y Z; Fan D M; Zhang X Y
CORPORATE SOURCE: Department of Gastroenterology, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, China.
SOURCE: World J Gastroenterol, (2001 Aug) 7 (4) 510-4.
Journal code: 100883448. ISSN: 1007-9327.
PUB. COUNTRY: China
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200206
ENTRY DATE: Entered STN: 20020308
Last Updated on STN: 20020606
Entered Medline: 20020605

AB AIM: To develop the single chain variable fragment of MG MG(7)murine

amplified separately and assembled into ***ScFv*** with a specially constructed DNA linker by PCR. The ScFvs DNA was ligated into the phagmid vector pCANTAB5E and the ligated sample was transformed into competent E.

phage to form MG MG(7) recombinant ***phage*** antibody library. The volume and recombinant rate of the library were evaluated by means of bacterial colony count and ***restriction*** analysis. After two rounds of panning with gastric cancer cell line KATO III of highly expressing MG(7)-binding antigen, the ***phage*** clones displaying ***ScFv*** of the antibody were selected by ELISA from the enriched ***phage*** clones. The antigen-binding affinity of the positive clone was detected by competition ELISA. HB2151 E. Coli was transfected with the positive ***phage*** clone demonstrated by competition ELISA for production of a soluble form of the MG(7) ***ScFv*** . ELISA assay was used to detect the antigen-binding affinity of the soluble MG(7) ***ScFv*** . Finally, the relative molecular mass of soluble MG(7) ***ScFv*** was measured by SDS-PAGE. RESULTS: The V(H), V(L) and ***ScFv*** DNAs were about 340bp, 320bp and 750bp, respectively. The volume of the library was up to 2 X 10(6) and 8 of 11 random clones were recombinants. Two ***phage*** clones could strongly compete with the original MG(7) antibody for binding to the antigen expressed on KATO III cells. Within 2 strong positive ***phage*** clones, the soluble MG(7) ***ScFv*** from one clone was found to have the binding activity with KATO III cells. SDS-PAGE showed that the relative molecular weight of soluble MG(7) ***ScFv*** was 32. CONCLUSION: The MG(7) ***ScFv*** was successfully produced by ***phage*** antibody technology, which may be useful for broadening the scope of application of the antibody.

L19 ANSWER 14 OF 51 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:461624 CAPLUS
DOCUMENT NUMBER: 136:182172
TITLE: Construction of anti-lipopolysaccharide single-chain ***phage*** antibody library in mice
AUTHOR(S): Chen, Ming; Fu, Weiling; Yu, Lili; Zhang, Xue
CORPORATE SOURCE: Department of Laboratory Diagnosis, Southwest Hospital, Third Military Medical University, Chungking, 400038, Peop. Rep. China
SOURCE: Di-San Junyi Daxue Xuebao (2001), 23(4), 407-409
CODEN: DYXUE8; ISSN: 1000-5404
PUBLISHER: Di-San Junyi Daxue
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB An anti-lipopolysaccharide (LPS) single chain ***phage*** antibody library in mice was constructed for further biomedical works. Total RNA was extd. from splenic cells for reverse transcription after BALB C mice immunized with pure LPS for 4 w. The designed primers were used to amplify the variable region genes of both heavy and light chain (VH, VL) with polymerase chain reaction. The VH and VL were then conjugated to form a single chain of variable fragment (***ScFv***) by a linker.

detect the exotic DNA. The titer of anti-LPS in murine sera was 1:12,800. The concn. of total RNA was 12.3812 .mu.g ml -1. The length of the fragments was 340 bp for VH, 320 bp for VL, and 800 bp for ***ScFv***

randomly selected clones was identified to contain the exotic DNA. The results showed that a 4.75 x 106 murine anti-LPS single chain ***phage*** antibody library was successfully constructed.

L19 ANSWER 15 OF 51 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 2001234486 MEDLINE
DOCUMENT NUMBER: 21109086 PubMed ID: 11182152
TITLE: A single-chain variable region immunoglobulin library from the abomasal lymph node of sheep infected with the gastrointestinal nematode parasite Haemonchus contortus.
AUTHOR: White G P; Meeusen E N; Newton S E
CORPORATE SOURCE: The Centre for Animal Biotechnology, School of Veterinary Science, The University of Melbourne, Parkville, 3052, Vic., Australia.. gregoryw@ichr.uwa.edu.au
SOURCE: VETERINARY IMMUNOLOGY AND IMMUNOPATHOLOGY, (2001 Jan 26) 78 (2) 117-29.
Journal code: 8002006. ISSN: 0165-2427.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010517
Last Updated on STN: 20010517
Entered Medline: 20010503

AB Sheep immunoglobulin (Ig) heavy-chain (V(H)DJ(H)) and lambda light-chain variable region (V(lambda)J(lambda)) nucleotide coding sequence was isolated by reverse transcriptase-polymerase chain reaction (RT-PCR) from abomasal lymph node (ALN) B cells of immune sheep challenged with the gastrointestinal nematode parasite Haemonchus contortus. Single-chain antibodies (***scFv***) were then constructed with the purified V(H)DJ(H) and V(lambda)J(lambda) Ig gene region DNA using oligonucleotides to PCR and join the variable regions to a central [Gly(4)Ser](3)-linker. In a similar fashion 5'-SfiI and 3'-NotI ***restriction*** endonuclease sites were added for cloning into a phagemid expression vector. Expression of sheep ***scFv*** from pHFA phagemid in an amber-suppresser strain of Escherichia coli, after infection with filamentous ***phage*** , resulted in 10(9) sheep ***scFv*** antibodies displayed as a library on phagemid particles. Western blot analysis demonstrated sheep ***scFv*** gene expression in E. coli cell lysate and on purified library ***phage*** . In addition, four rounds of ***scFv*** -library selection against H. contortus surface antigen resulted in a 300-fold increase in the elution titre of ***phage*** recovered from parasite surface antigen. Nearly 1000 of the selected and eluted scFvs were expressed in an attempt to identify monoclonal sheep

L19 ANSWER 16 OF 51 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:274166 CAPLUS

TITLE: Pertinence of kappa and lambda recombinant antibodies directed against thyroid peroxidase in thyroid autoimmune disease

AUTHOR(S): Bresson, Damien; Chardes, Thierry; Chapal, Nicolas; Bes, Cedric; Cerutti, Martine; Devauchelle, Gerard; Bouanani, Majida; Mani, Jean-Claude; Peraldi-Roux, Sylvie

CORPORATE SOURCE: CNRS-UMR 5094, Faculte de Pharmacie, Montpellier, 34060, Fr.

SOURCE: Human Antibodies (2001), 10(3,4), 109-118
CODEN: HUANFP; ISSN: 1093-2607

PUBLISHER: IOS Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Forty-one single-chain variable region fragments (scFvs) directed against thyroid peroxidase (TPO) were obtained by ***phage*** display libraries constructed from thyroid-infiltrating B cells of Graves' disease patients. Among these scFvs, 24.4% used a V.kappa. light chain whereas 75.6% shows a light chain of V.lambda. origin. Study of light chain gene usage in the TPO antibody repertoire demonstrated a dominance of the V.kappa.1-39 and V.lambda.1-51 genes. Thyroid peroxidase probing of overlapping peptides covering the amino acid sequences of anti-TPO T2/.kappa. and T12/.lambda. variable regions demonstrated a more ***restricted*** antigen recognition on T13/.lambda. than on T2/.kappa.. These two recombinant antibodies, expressed as whole IgG1 in the baculovirus/insect cell system, inhibited the binding to TPO of serum TPO autoantibodies whatever the light chain. Our study indicates that .lambda. as well as .kappa. light chain usage are found in the TPO antibody repertoire of thyroid-infiltrating B cells and are pertinent in the pathogenesis of autoimmune thyroid disease.

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L19 ANSWER 17 OF 51 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2001:477121 CAPLUS

DOCUMENT NUMBER: 136:145749

TITLE: Generation of antibody gene libraries from seropositive human donors

AUTHOR(S): Koch, Joachim; Dubel, Stefan

CORPORATE SOURCE: Universitat Heidelberg, Molekulare Genetik, Heidelberg, 69120, Germany

SOURCE: Antibody Engineering (2001), 109-123. Editor(s): Kontermann, Roland; Duebel, Stefan. Springer-Verlag: Berlin, Germany.
CODEN: 69BLB8

method is composed of four subprotocols. Subprotocol 1 is the polymerase chain reaction (PCR)-amplification of the rearranged variable light (.KAPPA., .lambda.) and variable heavy (.mu., .gamma.) antibody chains;

chain genes (1st PCR); subprotocol 3 is the introduction of ***restriction*** sites into the purified constructs of the first PCR reaction (2nd PCR); and subprotocol 4 is the cloning of the VH and VL chains into the expression vector pSEX81.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L19 ANSWER 18 OF 51 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 2001328433 MEDLINE
DOCUMENT NUMBER: 21289889 PubMed ID: 11395866
TITLE: The rescue by ***phage*** display of human Fabs to gp120 HIV-1 glycoprotein using EBV transformed lymphocytes.
AUTHOR: Kempf E; Weiss E; Klein P; Glacet A; Spratt S; Bourel D; Orfanoudakis G
CORPORATE SOURCE: Laboratoire d'immunotechnologie et microbiologie moleculaires, UPRES 1329, Ecole Superieure de Biotechnologie de Strasbourg, Boulevard Sebastien Brant, 67400 Illkirch, France.
SOURCE: MOLECULAR BIOTECHNOLOGY, (2001 Feb) 17 (2) 97-108. Journal code: 9423533. ISSN: 1073-6085.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200110
ENTRY DATE: Entered STN: 20011029
Last Updated on STN: 20011029
Entered Medline: 20011025

AB Human hybridomas secreting monoclonal antibodies in a stable manner are difficult to develop. The main difficulties are the ***restricted*** techniques for B-cell immortalization, the low number of sensitized B cells in peripheral blood, and the impossibility, for ethical reasons, to immunize humans with most antigens. ***Phage*** display has proved to be a powerful method for the generation of recombinant antibody fragments. This technology relies on the construction of recombinant Fab or ***scFv*** libraries and their display on ***phage*** M13. In order to rescue unstable B-cell clones secreting human antibodies we set up a method for the selection by ***phage*** display of human IgG fragments from Epstein-Barr virus (EBV)-transformed clones and applied it to the selection by ***phage*** display of Fabs directed against HIV-1 gp120, using a seropositive blood sample. The approach combines B-cell transformation by EBV of peripheral blood lymphocytes from a seropositive donor, preselection of specific IgG anti-gp120 producing clones, and the construction of a targeted human antibody library. In this library the percentage of heavy and light chain coding sequences expressed in

[illegible] efficiency of this technique.

L19 ANSWER 19 OF 51 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC
ACCESSION NUMBER: [illegible] BIOSIS

DOCUMENT NUMBER: PREV200200176305
TITLE: Streptococcus sanguis collagen-binding and collagen-like proteins identified by ***phage*** display.
AUTHOR(S): Kilic, A. O. (1); Herzberg, M. C.; Tao, L. (1)
CORPORATE SOURCE: (1) University of Illinois at Chicago, Chicago, IL USA
SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 47.
http://www.asmusa.org/mtgsrc/generalmeeting.htm. print.
Meeting Info.: 101st General Meeting of the American Society for Microbiology Orlando, FL, USA May 20-24, 2001
ISSN: 1060-2011.
DOCUMENT TYPE: Conference
LANGUAGE: English
AB It is well known that the oral commensal Streptococcus sanguis is a major pathogen in infective endocarditis. A predisposing factor for the disease is a defect in a heart valve. It is not clear, however, which surface proteins of this bacterium promote colonization on defective heart valves. Since collagen is a major connective tissue protein exposed on defective heart valves, this study sought to identify S. sanguis surface proteins that bind collagen and thus might promote colonization of heart valves. The ***phage*** display system, pG8SAET, was used to express S. sanguis proteins. Briefly, S. sanguis chromosomal DNA was digested with a combination of four ***restriction*** enzymes, SnaBI, RsaI, HincII, and EcoRV. The digested DNA fragments were inserted into the SnaBI cloning site of the pG8SAET phagemid by T4 ligase. The recombinant phagemid mixture was transformed into competent Escherichia coli strain TG1 cells to form a phagemid clone bank. The helper ***phage*** R408 (Promega) was used to infect the clone bank and generate a recombinant ***phage*** library. The phages were subjected to panning on a type I collagen-coated, 96-well microtiter plate. Non-adherent phages were removed by washing repeatedly with Na-citrate buffer (pH 5.5). The bound phages were eluted step-wise with the same buffer with decreasing pH (3.7 and 2.1). Three rounds of panning were performed. From the final collagen-bound recombinant phages, the DNA sequences of two clones were determined. BLAST analysis of the first S. sanguis gene suggested a possible collagenase. The second gene encodes an unknown protein that has a 14 amino acid domain identical to human osteocalcin and the mouse ***scFv*** collagenase IV antibody, and four domains (13 to 26 amino acids each) with 47-69% homology to human type V collagen. In conclusion, the identification of collagen-binding and collagen-like proteins of S. sanguis may suggest novel adhesion mechanisms for colonization of the tooth and damaged heart valves.

L19 ANSWER 20 OF 51 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:477116 CAPLUS

[redacted]

CORPORATE SOURCE: Universitat Zurich, Biochemisches Institut, Zurich, 8057, Switz.
SOURCE: Antibody Engineering (2001), 19-40. Editor(s):
[redacted]

Berlin, Germany.
CODEN: 69BLB8
DOCUMENT TYPE: Conference; General Review
LANGUAGE: English
AB A review. A prerequisite for the use of recombinant antibody technologies starting from hybridomas or immune repertories is the reliable cloning of functional Ig genes. For this purpose, a std. ***phage*** display system was optimized for robustness, vector stability, tight control of the expression of the ***scFv*** -geneIII fusion, primer usage for PCR amplification of variable region genes, ***scFv*** assembly strategy and subsequent directional cloning using a single rate cutting ***restriction*** enzyme. Using this system, a no. of variable antibody domains of hybridomas were accessible whose genes could not be cloned in previous expti. setups.
REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L19 ANSWER 21 OF 51 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:291578 CAPLUS
DOCUMENT NUMBER: 133:57236
TITLE: Characterization of superantigen-induced clonal deletion with a novel clan III- ***restricted*** avian monoclonal antibody: exploiting evolutionary distance to create antibodies specific for a conserved VH region surface
AUTHOR(S): Cary, Stephen P.; Lee, James; Wagenknecht, Raymond; Silverman, Gregg J.
CORPORATE SOURCE: The Sam and Rose Stein Institute for Research on Aging and the Theodore Gildred Cancer Center, Department of Medicine, University of California at San Diego, La Jolla, CA, 92093, USA
SOURCE: Journal of Immunology (2000), 164(9), 4730-4741
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Evolution of the Ab system has yielded three clans of VH region genes that are represented in almost every known higher species with an adaptive immune system. These clans are defined by sequence homologies primarily in highly conserved framework (FR) subdomains, which serve a scaffolding function maintaining the conformation of loops responsible for Ag binding. Structural analyses indicate that the VH FR1 and FR3 form a conserved composite exposed surface, which has been implicated in interactions with B cell superantigens. To directly investigate the expression of clan-defined supraclonal sets, the authors exploited the evolutionary

products, including those from the human VH3 family, and the analogous murine 7183, S107, J606, X24, and DNA4 families, and binding was competitive with natural B cell superantigens. The archetype, LJ-26, was

mammalian, and also the Xenopus and chicken, immune systems. In flow-cytometric studies with LJ-26, the authors found that treatment of heterozygous T15i transgenic mice with a model B cell superantigen induced a clan III- ***restricted*** clonal deletion. These studies demonstrate the utility of a novel recombinant serol. reagent to study the compn. of the B cell compartment and also the consequences of B cell superantigen exposure.

REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L19 ANSWER 22 OF 51 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:38776 CAPLUS
DOCUMENT NUMBER: 134:129959
TITLE: Panning of fibrin-specific single-chain antibodies from ***phage*** antibody library
AUTHOR(S): Sun, Lei; Gao, Xin-sheng; Huang, Yi-xiu; Zhu, Sheng-geng
CORPORATE SOURCE: College Life Sciences, Peking Univ., Beijing, 100871, Peop. Rep. China
SOURCE: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao (2000), 16(6), 722-726
CODEN: ZSHXF2; ISSN: 1007-7626
PUBLISHER: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB C ***phage*** antibody library was constructed in order to obtain human fibrin-specific antibody. MRNA was isolated from nonimmunized mouse spleens and used as the template for the first strand cDNA synthesis by reverse transcription primed with random hexamers. The variable region cDNA of antibody heavy and light chains (VH and VL) were amplified by polymerase chain reaction (PCR) sep. Both the VH and VL fragments were assembled into a single gene by using a DNA linker encoding a pentadecapeptide(Gly4Ser)3 through primary PCR. The assembled single-chain antibody (***ScFv***) DNA fragments were amplified with a set of oligonucleotide primers that introduce ***restriction*** sites for cloning via second PCR. The PCR product was digested with ***restriction*** enzymes and cloned into the phagmemid vector pCANTAB-5E. Then the recombinant DNA was introduced into Escherichia coli TG1 by electroporation. Rescue of recombinant ***phage*** antibody library was performed by superinfection of helper ***phage*** M13K07. The antibody library contains more than 1 .times. 108 ***phage*** ***ScFv*** antibodies. Using affinity selection or panning, the fibrin-specific ***ScFv*** displayed on the surface of fused phages was isolated from the ***phage*** antibody library. Imitating the

[redacted] whose sol. antibody proteins were expressed in E. coli strain HB2151, and then partially purified by Sephadex G-75 gel-filtration chromatog. The fibrin-specific ***ScFv*** could be useful in constructing directed [redacted]

L19 ANSWER 23 OF 51 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 2000214142 MEDLINE
DOCUMENT NUMBER: 20214142 PubMed ID: 10752477
TITLE: ***Phage*** -selected primate antibodies fused to superantigens for immunotherapy of malignant melanoma.
AUTHOR: Tordsson J M; Ohlsson L G; Abrahmsen L B; Karlstrom P J; Lando P A; Brodin T N
CORPORATE SOURCE: Active Biotech Research AB, Lund, Sweden.. Jesper.Tordsson@activebiotech.com
SOURCE CANCER IMMUNOLOGY, IMMUNOTHERAPY, (2000 Mar) 48 (12) 691-702.
Journal code: 8605732. ISSN: 0340-7004.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000427
Last Updated on STN: 20000427
Entered Medline: 20000419

AB The high-molecular-weight melanoma-associated antigen, HMW-MAA, has been demonstrated to be of potential interest for diagnosis and treatment of malignant melanoma. Murine monoclonal antibodies (mAb) generated in response to different epitopes of this cell-surface molecule efficiently localise to metastatic lesions in patients with disseminated disease. In this work, ***phage*** -display-driven selection for melanoma-reactive antibodies generated HMW-MAA specificities capable of targeting bacterial superantigens (SAg) and cytotoxic T cells to melanoma cells. Cynomolgus monkeys were immunised with a crude suspension of metastatic melanoma. A strong serological response towards HMW-MAA demonstrated its role as an immunodominant molecule in the primate. Several clones producing monoclonal ***scFv*** antibody fragments that react with HMW-MAA were identified using melanoma cells and tissue sections for ***phage*** selection of a recombinant antibody ***phage*** library generated from lymph node mRNA. One of these ***scFv*** fragments, K305, was transferred and expressed as a Fab-SAg fusion protein and evaluated as the tumour-targeting moiety for superantigen-based immunotherapy. It binds with high affinity to a unique human-specific epitope on the HMW-MAA, and demonstrates more ***restricted*** cross-reactivity with normal smooth-muscle cells than previously described murine mAb. The K305 Fab was fused to the superantigen staphylococcal enterotoxin A (D227A) [SEA(D227A)], which had been mutated to reduce its intrinsic MHC class II binding affinity, and the fusion protein was used to demonstrate redirection of T cell cytotoxicity to melanoma cells in vitro. In mice

[redacted] high-affinity binding and selectivity, supporting its use for tumour therapy in conjunction with T-cell-activating superantigens.

[redacted]

ACCESSION NUMBER: 2000404385 MEDLINE
DOCUMENT NUMBER: 20378952 PubMed ID: 10918198
TITLE: A3--a novel colon and pancreatic cancer reactive antibody from a primate ***phage*** library selected using intact tumour cells.
AUTHOR: Tordsson J; Lavasani S; Ohlsson L; Karlstrom P; Svedberg H; Abrahmsen L; Brodin T
CORPORATE SOURCE: Active Biotech Research AB, Lund, Sweden.. Jesper.Tordsson@Activebiotech.com
SOURCE: INTERNATIONAL JOURNAL OF CANCER. (2000 Aug 15) 87 (4) 559-68.
Journal code: 0042124 ISSN: 0020-7136.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000901
Last Updated on STN: 20000901
Entered Medline: 20000818
AB The identification of novel tumour-associated antigens (TAAs) is pivotal for progression in the fields of tumour immunotherapy and diagnosis. In the present study, we have developed, based on flow cytometric evaluation and use of a mini-library composed of specific antibody clones linked to different antibiotic resistance markers, methods for positive and subtractive selection of ***phage*** antibodies employing intact cells as the antigen source. An ***scFv*** ***phage*** library (2.7 x 10(7)) was constructed from a primate (Macaca fascicularis) immunised with pooled human colon carcinomas. This library was selected for 3 rounds by binding to Colo 205 colon adenocarcinoma cells and proteolytic elution followed by ***phage*** amplification. Several antibodies reactive with colon carcinomas and with ***restricted*** reactivity to a few epithelial normal tissues were identified by immunohistochemistry. One clone, A3 ***scFv*** , recognised an epitope that was homogeneously expressed in 11/11 of colon and 4/4 pancreatic carcinomas studied and in normal tissue ***restricted*** to subtypes of epithelia in the gastrointestinal tract. The A3 ***scFv*** had an apparent overall affinity approximately 100-fold higher than an A3 Fab, suggesting binding of ***scFv*** homodimers. The cell surface density of the A3 epitope, calculated on the basis of Fab binding, was exceptionally high, approaching 3 million per cell. We also demonstrate efficient T-cell-mediated killing of colon cancer cells coated with A3 ***scFv*** fused to the low MHC class II binding superantigen mutant SEA(D227A). The identified A3 molecule thus represents a TAA with properties that suggest its use for immunotherapy of colon and pancreatic cancer.

ACCESSION NUMBER: [illegible]
DOCUMENT NUMBER: 135,93
TITLE: Construction and expression of ***phage*** display single chain antibody ***scFv*** library from

AUTHOR(S): Sui, Jianhua; Song, Zengxuan; She, Ming; Zhang, Liyan; Shen, Decheng; Han, Zhongchao
CORPORATE SOURCE: Institute of Hematolgy, Chinese Academy of Medical Science + Peking Union Medical College, Tianjin, 300020, Peop. Rep. China
SOURCE: Zhongguo Mianyixue Zazhi (2000), 16(6), 318-321
CODEN: ZMZAEE; ISSN: 1000-484X
PUBLISHER: Zhongguo Mianyixue Zazhi Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB A single chain antibody ***scFv*** library was constructed by ***phage*** display technique from the spleen cells of mice immunized with KG1a cells. Three mice were immunized with KGla cells, their spleen cells were isolated. The genes of VH and Vk were amplified by RT-PCR and a ***scFv*** - ***phage*** display antibody library was constructed with the amplified V gene, and the expression of the library were examd. by SDS-PAGE A ***scFv*** library contg. 3 x 106 individual clones was obtained and showed different patterns after digested with ***restriction*** endonuclease BstN I. The capacity and diversity of the library were sufficient for screening specific ***scFv*** . The surface display expression of the library was verified. The ***scFv*** library of anti-KGla cell surface mols. may be used for isolating specific ***scFv*** against the cell surface mols. of hematopoietic progenitors.

L19 ANSWER 26 OF 51 MEDLINE DUPLICATE 10
ACCESSION NUMBER 2000192069 MEDLINE
DOCUMENT NUMBER: 20192069 PubMed ID: 10725458
TITLE: A rapid and versatile method for harnessing ***scFv*** antibody fragments with various biological effector functions.
AUTHOR: Helfrich W; Haisma H J; Magdolen V; Luther T; Bom V J; Westra J; van der Hoeven R; Kroesen B J; Molema G; de Leij L
CORPORATE SOURCE Groningen University Institute for Drug Exploration (GUIDE) at the University Hospital Groningen, Department of Pathology and Laboratory Medicine, Medical Biology Branch, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands.. w.helfrich@med.rug.nl
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (2000 Apr 3) 237 (1-2) 131-45.
Journal code: 1305440. ISSN: 0022-1759.
PUB. COUNTRY Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

AB A versatile expression vector is described for the rapid construction and evaluation of bispecific scFvs and ***scFv*** -based fusion proteins. An important feature of this vector is the presence of two multiple

MCS was specifically designed to contain unique SfiI and NotI ***restriction*** enzyme sites that can be used for directional and in frame insertion of scFvs (or potentially any molecule) selected from established ***phage*** -display systems. Using this new vector, a functional bs-(***scFv***)(2) (2C11-MOC31) was constructed for retargeted T-cell cytotoxicity towards EGP2 positive tumor cells. The vector was also used for grafting of a number of promising biological effector principles onto ***scFv*** MOC31, including the prodrug converting enzyme cytosine deaminase, the anti-angiogenic factor angiostatin, and the thrombogenic molecule tissue factor. We aimed at producing biologically active fusion proteins by directing them through the endoplasmic reticulum-based protein folding machinery of eukaryotic cells (COS-7) using a kappa light chain leader, thereby taking advantage of the associated quality control mechanisms that allow only fully folded and processed fusion proteins to be secreted into the medium. Supernatants derived from fusion protein transfected COS-7 cells, which were transiently transfected at low transfection rates, were directly assayed for the biological and/or targeting activity of the excreted fusion proteins without any prior purification steps. This procedure might help to identify those fusion proteins that have favourable characteristics like stability and biological activity in the presence of serum and at low protein concentrations. Targeted delivery of all effector principles was subsequently assessed in an in vitro model system. The method we devised is both rapid and versatile and can be useful to construct and identify series of new chimeric proteins with enhanced therapeutic potential in human cancer therapy.

L19 ANSWER 27 OF 51 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 2000164762 MEDLINE
DOCUMENT NUMBER: 20164762 PubMed ID: 10699585
TITLE: Guinea pig C3 specific rabbit single chain Fv antibodies from bone marrow, spleen and blood derived ***phage*** libraries.
AUTHOR: Hawlisch H; Meyer zu Vilsendorf A; Bautsch W; Klos A; Kohl J
CORPORATE SOURCE: Institute of Medical Microbiology, Medical School Hannover, Hannover, Germany.
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (2000 Mar 6) 236 (1-2) 117-31.
Journal code: 1305440. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200005

rabbit antibody repertoire of bone marrow, spleen and peripheral blood of a rabbit immunized with guinea pig complement protein C3. By means of the ***phage*** display technology we selected guinea pig C3 specific single

the ***scFv*** antibodies cross reacted with guinea pig C3a, human C3 or rat C3. The frequency of bone marrow derived C3 positive clones was much higher as compared to blood or spleen derived clones. Additionally bone marrow and spleen derived clones show higher diversity than clones, obtained from blood, as determined by fingerprint analysis with the ***restriction*** enzyme AluI. Dissociation rate constants for all scFvs were similar, indicating that the source of the scFvs had no influence on affinities. The antibody fragments were used to analyze complement activation during xenotransplantation. Several blood or bone marrow derived scFvs bound to C3 located on rat liver endothelium after hyperacute rejection of a heterotopically transplanted rat liver into guinea pig. These data demonstrate that monoclonal rabbit scFvs can be easily generated from recombinant ***phage*** display libraries, constructed from spleen, blood or bone marrow. The selected guinea pig C3 specific scFvs appear to be useful to detect complement activation during xenotransplantation in guinea pigs.

L19 ANSWER 28 OF 51 MEDLINE
ACCESSION NUMBER: 1999145601 MEDLINE
DOCUMENT NUMBER: 99145601 PubMed ID: 9990075
TITLE: Human single-chain Fv immunoconjugates targeted to a melanoma-associated chondroitin sulfate proteoglycan mediate specific lysis of human melanoma cells by natural killer cells and complement.
AUTHOR: Wang B; Chen Y B; Ayalon O; Bender J; Garen A
CORPORATE SOURCE: Department of Molecular Biophysics and Biochemistry Yale University, New Haven, CT 06520, USA.
CONTRACT NUMBER: RO1 HL43331 (NHLBI)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 Feb 16) 96 (4) 1627-32. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990402
Last Updated on STN: 19990402
Entered Medline: 19990325

AB Two antimelanoma immunoconjugates containing a human single-chain Fv (***scFv***) targeting domain conjugated to the Fc effector domain of human IgG1 were synthesized as secreted two-chain molecules in Chinese hamster ovary and Drosophila S2 cells, and purified by affinity chromatography on protein A. The ***scFv*** targeting domains originally were isolated as melanoma-specific clones from a ***scFv***

[redacted]

several other types of normal cells and tumor cells. A 250-kDa melanoma protein was immunoprecipitated by the immunoconjugates and analyzed by mass spectrometry, using two independent procedures. A screen of protein

[redacted]

the immunoprecipitated protein and the core protein of a chondroitin sulfate proteoglycan, which is expressed on the surface of most human melanoma cells. The Fc effector domain of the immunoconjugates binds natural killer (NK) cells and also the C1q protein that initiates the complement cascade; both NK cells and complement can activate powerful cytolytic responses against the targeted tumor cells. An in vitro cytolysis assay was used to test for an immunoconjugate-dependent specific cytolytic response against cultured human melanoma cells by NK cells and complement. The melanoma cells, but not the human fibroblast cells used as the control, were efficiently lysed by both NK cells and complement in the presence of the immunoconjugates. The in vitro results suggest that the immunoconjugates also could activate a specific cytolytic immune response against melanoma tumors in vivo.

L19 ANSWER 29 OF 51 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 12

ACCESSION NUMBER: 2000 27481 BIOSIS

DOCUMENT NUMBER: PREV200000027481

TITLE: Isolation of ***ScFv*** antibodies of rP27Kip1 from ***phage*** display libraries constructed from immunized and non-immunized repertoires.

AUTHOR(S): Cao Yueqiong (1); Qiao Shouyi (1); Yuan Youzhong; Huang Jiansheng (1); Zhao Shouyuan (1)

CORPORATE SOURCE: (1) State Key Laboratory of Genetic Engineering, Institute of Genetics, Fudan University, Shanghai, 200433 China

SOURCE: Science in China Series C Life Sciences, (Aug., 1999) Vol. 42, No. 4, pp. 376-382.
ISSN: 1006-9305.

DOCUMENT TYPE: Article

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Through mRNA extract, RT and a series of PCR, ***phage*** antibody libraries were constructed from rP27Kip1-immunized and non-immunized mice. After only one round of selection with rP27Kip1, clones from each library were chosen randomly and digested by Taq I and Hinf I. 11 of 64 clones from the immunized animal had consistent ***restriction*** pattern, while none of the 64 clones from the non-immunized animal had, except that one had the same fragments pattern as that of the 11 clones. The 12 fragments were expressed in E. coli BL21(DE3) pET-28b(+) system. ELISA showed that some of the fragments could bind to rP27Kip1 specifically. All these results implied that specific antibody can be obtained by genetic engineering without hybridoma or many rounds of growth and panning selection.

L19 ANSWER 30 OF 51 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC

modified to contain a Sfi I and Not I site

AUTHOR(S): Yi, Kyesook; Chung, Junho; Kim, Hyojung; Kim, Ikjung; Jung, Hyanggi; Kim, Jungran; Choi, Inhak; Suh, Pannghill; Chung,

CORPORATE SOURCE: (1) Department of Biochemistry, College of Medicine, Seoul National University, Seoul, 110-799 South Korea
SOURCE: Hybridoma, (June, 1999) Vol. 18, No. 3, pp. 243-249.
ISSN: 0272-457X.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The CEA 79 antibody has been used in bone marrow scintigraphy for the differential diagnosis of skeletal tumors and the evaluation of the bone marrow status of patients with various hematological disorders. The specific localization of radio-labeled CEA 79 antibody in bone marrow depends on its reactivity with NCA-95 (nonspecific cross-reacting antigen-95) present on the surface and in the cytosol of human granulocytes and myelopoietic cells. To make a CEA 79 ***scFv*** molecule that would be less immunogenic and more penetrating than the intact mouse immunoglobulin, we constructed a pRSET Sfi I/Not I expression vector. The ***scFv*** gene was then excised from a pCANTAB 5 E ***phage*** display vector by digestion with Sfi I and Not I and inserted into the pRSET Sfi I/Not I expression vector. Upon transformation of a BL21(DE3)pLysS strain of E. coli, CEA 79 ***scFv*** became expressed in inclusion bodies requiring a renaturation process for solubilization. The final yield of CEA 79 ***scFv*** was 5 mg per a literof culture. The refolded CEA 79 ***scFv*** exhibited an affinity (Kd = 2.1 X 10-9 M) equivalent to that of the original CEA 79 antibody (Kd - 3.3 X 10-9 M) and the same immunoreactivity to CEA and NCA-95 in Western blots and in immunohistochemical staining experiments.

L19 ANSWER 31 OF 51 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:764702 CAPLUS
DOCUMENT NUMBER: 132:203779
TITLE: Screening and sequencing of single-chain antibody ***ScFv*** genes of three recombined phages with G-CSF binding activity
AUTHOR(S): Jia, Songhui; Guo, Yao; Fan, Ling-Zhi; Liang, Mifang; Hou, Yunde
CORPORATE SOURCE: Department of pathophysiology, The Fourth Military Medical University, Xi'an, 710032, Peop. Rep. China
SOURCE: Xibao Yu Fenzi Mianyixue Zazhi (1999), 15(3), 161-164
CODEN: XFMZFM; ISSN: 1007-8738
PUBLISHER: Disi Junyi Daxue
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB Using binding to solid phase G-CSF followed by elution and re-infection, we isolated ***ScFv*** phagemid clones from a ***phage*** display library contg. anti-human G-CSF repertoire ***ScFv*** gene. After

confirmed that their sequences belonged to the mouse Ig V-region genes. They appeared to be novel genes by comparison with published data in EMBI GeneBank.

L19 ANSWER 32 OF 51 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 2000027002 MEDLINE
DOCUMENT NUMBER: 20027002 PubMed ID: 10556547
TITLE: Selection of ***phage*** antibodies to surface epitopes of Phytophthora infestans.
AUTHOR: Gough K C; Li Y; Vaughan T J; Williams A J; Cockburn W; Whitelam G C
CORPORATE SOURCE: Department of Biology, Adrian Building, University of Leicester, University Road, Leicester, UK.
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (1999 Aug 31) 228 (1-2) 97-108.
Journal code: 1305440. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991228

AB Antibodies specific for surface-exposed epitopes on germlings of the plant pathogen, Phytophthora infestans, were isolated from a diverse ***phage*** library displaying single-chain Fv (***scFv***) antibody fragments. The library was subpanned against external soluble components released from mycelia, sporangia and germlings and a discrete population of ***phage*** antibodies isolated. Binding of monoclonal ***phage*** antibodies was demonstrated by enzyme-linked immunosorbent assay (ELISA) and diversity was established by BstNI ***restriction*** enzyme digest patterns. Antibodies were subcloned as fusions at the C-terminus of maltose binding protein (MBP) and expressed as soluble proteins in Escherichia coli. These antibody fusion proteins bound to P. infestans germlings and to mycelial homogenates from various Phytophthora species. The binding activities to mycelial homogenates of fungal species not belonging to the order Peronosporales were substantially lower. Several ***phage*** -displayed scFvs were used in conjunction with fluorescently labelled antiphage antibody to visualise the distribution of their cognate epitopes on the surface of the germlings. The combination of procedures developed here with Phytophthora demonstrates the potential of ***phage*** antibody technology in isolating antibodies to cell surface and external soluble components of pathogens, some of which may play a role in host pathogen interactions.

L19 ANSWER 33 OF 51 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 1999288214 MEDLINE
DOCUMENT NUMBER: 99288214 PubMed ID: 10336861

[illegible]
CORPORATE SOURCE: Laboratory for Monoclonal Antibodies, Wageningen Agricultural University, Wageningen, 6700 ES, The Netherlands.
[illegible]

63-9.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF083070
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990727
Last Updated on STN: 19990727
Entered Medline: 19990712

AB The vector pSKAP/S was constructed to enable overexpression of single-chain variable fragment antibody (***scFv***)-alkaline phosphatase fusion proteins. In pSKAP/S, the ***scFv*** were genetically fused to the mutated Escherichia coli PhoA/S gene that encodes an alkaline phosphatase with increased specific activity. The ***restriction*** sites incorporated into pSKAP/S allowed the ***scFv*** genes to be easily transferred from pUC119-derived phagemid vectors that are used frequently in ***phage*** display antibody library technology. Strong transcriptional control of expression was achieved using the tetracycline promoter, and induction of different individual clones with anhydrotetracycline resulted in secretion of most of the ***scFv*** -alkaline phosphatase fusion proteins into the culture medium. Although some of the clones secreted fusion proteins that were retained in the periplasm, these proteins could be isolated with a simple extraction procedure. Increased amounts of a ***scFv*** -alkaline phosphatase fusion protein were obtained when expressed in the pSKAP/S vector compared with expression in a vector incorporating the lac promoter. Testing for binding of the ***scFv*** -alkaline phosphatase fusion proteins to antigen was possible in an ELISA without the need for additional enzyme-conjugated antibodies. The pSKAP/S vector was successfully used to obtain ***scFv*** fragments from a preparation of ***phage*** -antibody clones after subcloning and expression of individual clones as ***scFv*** -alkaline phosphatase fusions, whereas fewer clones (and clones with different properties) were obtained from the same ***phage*** -antibody preparations when expressed as soluble ***scFv*** fragments. Therefore, the pSKAP/S vector was shown to be useful in extending the range of ***scFv*** obtained from ***phage*** display libraries.
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L19 ANSWER 34 OF 51 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:620743 CAPLUS
DOCUMENT NUMBER: 131:321211
TITLE: Dissecting the human peripheral B-cell compartment

[redacted]

SOURCE: Immunology (1999), 98(1), 55-62
CODEN: IMMUAM; ISSN: 0019-2805
PUBLISHER: Blackwell Science Ltd
[redacted]

LANGUAGE: English

AB Previously we have employed a large semisynthetic ***phage*** antibody display library, in combination with subtractive selection by flow cytometry to isolate ***phage*** antibodies specific for subpopulations of leukocytes. In this study, human tonsillar B cells were incubated with the ***phage*** library and IgD- CD38- memory B lymphocytes and attached ***phage*** antibodies were selected by cell sorting. In a panel of 17 monoclonal ***phage*** antibodies obtained, five displayed binding to cells of multiple haematopoietic lineages or broadly reacted with B-lineage cells. Immunofluorescent, immunohistochem. and biochem. studies permitted the characterization of the target mols. recognized by these ***phage*** antibodies. The remaining 12 antibodies displayed ***restricted*** binding to small subpopulations of peripheral human B cells. These results show that subtractive selections with ***phage*** antibody display libraries in combination with flow cytometry yield antibodies that bind to differentially expressed mols. on closely related cell populations, and can be used as a tool in a variety of assays.

REFERENCE COUNT: 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L19 ANSWER 35 OF 51 MEDLINE DUPLICATE 15

ACCESSION NUMBER: 1999226765 MEDLINE

DOCUMENT NUMBER: 99226765 PubMed ID: 10211783

TITLE: ***Phage*** library-derived human anti-TETA and anti-DOTA ***ScFv*** for pretargeting RIT.

AUTHOR: DeNardo S J; DeNardo G L; Brush J; Carter P

CORPORATE SOURCE: Department of Internal Medicine, University of California Davis Medical Center, Sacramento 95816, USA.

CONTRACT NUMBER: CA47829 (NCI)

SOURCE: HYBRIDOMA, (1999 Feb) 18 (1) 13-21.
Journal code: 8202424. ISSN: 0272-457X.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199908

ENTRY DATE: Entered STN: 19990827
Last Updated on STN: 19990827
Entered Medline: 19990819

AB Pretargeting techniques have promise for radioimmunotherapy (RIT) in cancer because of the potential for markedly increasing the therapeutic ratio. Human antibody fragments can be retrieved from ***phage*** libraries and used to realize this potential. The library can be used to select the genetic material required to generate molecules with binding

[redacted]

bispecific pretargeting molecules and to bind radiochelates given subsequently as Cu-67-TETA or Y-90-DOTA. ***Phage*** that displayed the anti-chelate ***scFv*** were selected by absorption to antibody

[redacted]

(ELISA) were performed to assess the intensity and specificity of ***phage*** binding to the specific chelate. Ninety-six clones demonstrating metal chelate binding seven times greater than to Lym-1 alone were chosen for diversity analysis. BstN I ***restriction*** digests were performed on DNA from these clones. Twenty-three and 43 different DNA fingerprint patterns were identified for anti-TETA and anti-DOTA clones, respectively. DNA sequencing of 39 anti-TETA clones for 23 different BstN I fingerprint patterns revealed 22 distinct sequences. Eleven of the anti-TETA clones were selected for further study. Five hundred to 1000 microg (100 to 320 microg per liter of culture) of purified ***scFv*** was produced from each of the 11 anti-TETA clones. Preliminary studies by BIAcore demonstrated evidence of 25- to 200-nM affinities. Comparable examination of the anti-DOTA clones is in progress. This study provides evidence that human ***scFv*** against unique synthetic targets can be readily selected from a large, naive human immunoglobulin ***phage*** library. Selections against metal chelated antibodies provided a wealth of scFvs with diverse binding affinities useful for engineering molecules for pretargeting RIT.

L19 ANSWER 36 OF 51 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 16

ACCESSION NUMBER: 1999:281561 BIOSIS

DOCUMENT NUMBER: PREV199900281561

TITLE: Selection of rabbit single-chain Fv fragments against the herbicide atrazine using a new ***phage*** display system.

AUTHOR(S): Li, Yi (1); Cockburn, William; Kilpatrick, John; Whitelam, Garry C.

CORPORATE SOURCE: (1) Department of Biology, University of Leicester, Leicester, LE1 7RH UK

SOURCE: Food and Agricultural Immunology, (March, 1999) Vol. 11, No. 1, pp. 5-17.
ISSN: 0954-0105.

DOCUMENT TYPE: Article

LANGUAGE: English

SUMMARY LANGUAGE: English

AB A convenient new bacteriophage display vector, pSD3, has been constructed and used to generate rabbit monoclonal anti-pesticide antibody fragments. Following amplification of immunoglobulin light chain, and heavy chain variable region gene libraries, ***restriction*** enzymes Sfi I and Prim I are used to assemble ***scFv*** libraries in pSD3. This allows the number of stages involving the polymerase chain reaction and ***restriction*** enzyme digestion to be minimized to optimize maintenance of the original diversity of the variable region genes in the libraries. The vector also incorporates an amber codon, a 6xHis tag and a

[redacted] of single-chain Fvs by competition and equilibrium FIFSA indicated good specificity and affinity to atrazine.

ACCESSION NUMBER: 1998414292 MEDLINE
DOCUMENT NUMBER: 98414292 PubMed ID: 9743360
TITLE: Limited diversity of human ***scFv*** fragments isolated by panning a synthetic ***phage*** -display ***scFv*** library with cultured human melanoma cells.
AUTHOR: Noronha E J; Wang X; Desai S A; Kageshita T; Ferrone S
CORPORATE SOURCE: Department of Microbiology and Immunology, New York Medical College, Valhalla 10595, USA.
CONTRACT NUMBER: CA37959 (NCI)
CA51814 (NCI)
SOURCE: JOURNAL OF IMMUNOLOGY, (1998 Sep 15) 161 (6) 2968-76.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19981020
Last Updated on STN: 19981020
Entered Medline: 19981006
AB To broaden the specificity of the Abs recognizing human melanoma-associated Ags (MAAs), we have isolated human single-chain fragment of the V region (***scFv***) fragments by panning the synthetic ***phage*** Ab library (#1) with the human melanoma cell lines S5 and SK-MEL-28. All of the isolated ***scFv*** fragments reacted with the mouse mAb defined high molecular weight melanoma-associated Ag (HMW-MAA). ***scFv*** #70 immunoprecipitates the two characteristic subunits of HMW-MAA, while ***scFv*** #28 only immunoprecipitates its large subunit. These results challenge the current view regarding the structure of HMW-MAA and indicate that it consists of two independent subunits. The human ***scFv*** fragments share some similarities with the mouse anti-HMW-MAA mAb. Like mAb 149.53 and 225.28, ***scFv*** #28 reacts with rat B49 neural cells that express a homologue of HMW-MAA. ***scFv*** #70 reacts with a determinant that is spatially close to the one identified by mAbs 149.53, VT68.2, and VT86. Besides suggesting similarities in the recognition of human melanoma cells by the mouse and human Ab repertoire, these results indicate that the Abs isolated from synthetic Ab libraries resemble those that are found in natural Ab repertoires. The ***restricted*** diversity of the ***scFv*** fragments that were isolated by panning synthetic Ab libraries with different melanoma cell lines suggests that certain Ags, like HMW-MAA, are immunodominant in vitro. This phenomenon, which parallels the in vivo immunodominance of certain Ags, implies that the antigenic profile of the cells used for panning determines the specificity of the preponderant population of isolated Abs.

[illegible]

TITLE: Characterization of human anti-high molecular weight-melanoma-associated antigen single-chain Fv fragments isolated from a ***phage*** display antibody
[illegible]

Untitled

AUTHOR Desai S A; Wang X; Noronha E J; Kageshita T; Ferrone S
CORPORATE SOURCE: Department of Microbiology and Immunology, New York Medical College, Valhalla 10595, USA.
CONTRACT NUMBER: CA37959 (NCI)
CA51814 (NCI)
SOURCE: CANCER RESEARCH, (1998 Jun 1) 58 (11) 2417-25.
Journal code: 2984705R. ISSN: 0008-5472.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980716
Last Updated on STN: 19980716
Entered Medline: 19980707

AB The human high molecular weight-melanoma-associated antigen (HMW-MAA) meets the criteria to be used as an immunogen for immunotherapy of malignant melanoma, because it is expressed by a large percentage of melanoma lesions with limited heterogeneity and has a ***restricted*** distribution in normal tissues. The high immunogenicity of the HMW-MAA in BALB/c mice has resulted in the development of a large number of anti-HMW-MAA monoclonal antibodies (mAbs). In contrast, no human anti-HMW-MAA mAbs have been described. Because the latter may serve as useful probes to characterize the antigenic profile of the HMW-MAA, human anti-HMW-MAA single-chain fragments of the variable region (scFvs) were isolated by panning synthetic ***scFv*** library 1 on purified HMW-MAA. Colony hybridization studies and nucleotide sequence analysis revealed that ***scFv*** 19, 44, 56, and 61 belong to the V(H)3 gene family and use the DP-38 germ-line gene segment but have a diverse third complementarity-determining region. The human scFvs share some characteristics with mouse anti-HMW-MAA mAb but also display some distinct features. Like mouse mAbs, human scFvs recognize determinants of HMW-MAA with a heterogeneous cellular and molecular distribution in human melanoma cells. Furthermore, like some mouse mAbs, human scFvs react with rat neural cells expressing the chondroitin sulfate proteoglycan NG2, which shows 81% homology to the HMW-MAA. However, at variance with mouse mAbs, the human scFvs show poor reactivity with guinea pig melanoma cells. Lastly, human ***scFv*** 61 stains both benign and malignant lesions of melanocytic origin, although with a lower frequency than mouse mAbs. Analysis of the clinical significance of the differential expression of the ***scFv*** 61-defined determinant in melanoma lesions will be facilitated by its reactivity with formalin-fixed melanoma lesions. In contrast to mouse mAb, ***scFv*** 61 immunoprecipitates the 450-kDa chondroitin sulfate proteoglycan component of the HMW-MAA, but not its 250-kDa subunit from melanoma cells. Thus, contrary to the current view

studies will assess the impact of these in vitro-assembled antibody fragments on the identification of antigenic determinants of the HMW-MAA that can be recognized by the human immune system

L19 ANSWER 39 OF 51 MEDLINE DUPLICATE 19
ACCESSION NUMBER 1998325681 MEDLINE
DOCUMENT NUMBER: 98325681 PubMed ID: 9661204
TITLE: An efficient route to human bispecific IgG.
AUTHOR: Merchant A M; Zhu Z; Yuan J Q; Goddard A; Adams C W; Presta L G; Carter P
CORPORATE SOURCE Department of Molecular Oncology, Genentech Inc., South San Francisco, CA 94080, USA.
SOURCE: NATURE BIOTECHNOLOGY, (1998 Jul) 16 (7) 677-81.
Journal code: 9604648. ISSN: 1087-0156.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF048774; GENBANK-AF048775
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19981021
Last Updated on STN: 20000303
Entered Medline: 19981015
AB Production of bispecific IgG (BsIgG) by coexpressing two different antibodies is inefficient due to unwanted pairings of the component heavy and light chains. To overcome this problem, heavy chains were remodeled for heterodimerization using engineered disulfide bonds in combination with previously identified "knobs-into-holes" mutations. One of the variants, S354C:T366W/Y349'C:T366'S:L368'A:Y407++ +'V, gave near quantitative (approximately 95%) heterodimerization. Light chain mispairing was circumvented by using an identical light chain for each arm of the BsIgG. Antibodies with identical light chains that bind to different antigens were identified from an ***scFv*** ***phage*** library with a very ***restricted*** light chain repertoire for the majority (50/55) of antigen pairs tested. A BsIgG capable of simultaneously binding to the human receptors HER3 and cMpl was prepared by coexpressing the common light chain and corresponding remodeled heavy chains followed by protein A chromatography. The engineered heavy chains retain their ability to support antibody-dependent cell-mediated cytotoxicity as demonstrated with an anti-HER2 antibody.

L19 ANSWER 40 OF 51 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:228196 CAPLUS
DOCUMENT NUMBER: 128:304548
TITLE: Construction of gene for single-chain Fv (***scFv***) abzyme with glutathione peroxidase activity
AUTHOR(S): Feng, Shuzhang; Liu, Zi; Guo, Xuejun; Zhang, Guoli; Wu, Guangmu; Luo, Guimin; Gao, Shujuan
CORPORATE SOURCE: Univ. of Agric. and Anim. Sci., Mil. Vet. Inst.,

DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB Following a chem mutation, the monoclonal antibody secreted by mouse

3.6 times as high as that of native glutathione peroxidase from rabbit liver. The gene for the single-chain Fv (***scFv***) abzyme was successfully constructed by total RNA and mRNA isolation, cDNA synthesis, VH and VL PCR amplification and assembly. On agarose gel electrophoresis the lengths of VH, VL and ***ScFv*** gene fragments were 340, 325 and 750 bp resp. The ***ScFv*** gene with terminal ***restriction*** site SfiI and NotI was considered to be used for ***ScFv*** abzyme expression in recombinant ***phage*** antibody system.

L19 ANSWER 41 OF 51 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:717997 CAPLUS
DOCUMENT NUMBER: 128:1458
TITLE: Isolation of enzymes using enzyme-calmodulin fusion proteins and substrate binding
INVENTOR(S): Neri, Dario; Demartis, Salvatore; Huber, Adrian; Viti, Francesca; Tawfik, Dan S.; Winter, Gregory Paul
PATENT ASSIGNEE(S): Medical Research Council, UK; Neri, Dario; Demartis, Salvatore; Huber, Adrian; Viti, Francesca; Tawfik, Dan S.; Winter, Gregory Paul
SOURCE: PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9740141	A2	19971030	WO 1997-GB1153	19970425
WO 9740141	A3	19980108		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2250922	AA	19971030	CA 1997-2250922	19970425
AU 9726446	A1	19971112	AU 1997-26446	19970425
AU 721683	B2	20000713		
EP 895536	A2	19990210	EP 1997-918248	19970425
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000508905	T2	20000718	JP 1997-537862	19970425

[illegible]

desired chemical or biological activity, the method comprising linking the enzyme to a substrate for the enzyme, reacting the enzyme and substrate such that a product is produced which remains linked to the enzyme and isolating the [illegible]

vector plagemid, pDN323, was designed which contained a polylinker region (including ***restriction*** sites for NcoI, PstI, XhoI, and NotI), a calmodulin gene, a filamentous ***phage*** tail protein pIII gene, and gene pelB. Expression of phagemid pDN323 produced a fusion protein contg. pectin lyase B, calmodulin and protein pIII. A synthetic peptide (for example: CGGAAARWKKAFIAVSAANRFKKIS) was used to bind the calmodulin in the fusion protein. Examples of selection of other enzymes include insulin receptor tyrosine kinase, Lck tyrosine kinase, and glutathione S-transferase.

L19 ANSWER 42 OF 51 MEDLINE DUPLICATE 20
ACCESSION NUMBER: 1998023107 MEDLINE
DOCUMENT NUMBER: 98023107 PubMed ID: 9358270
TITLE: A TNF receptor antagonistic ***scFv*** , which is not secreted in mammalian cells, is expressed as a soluble mono- and bivalent ***scFv*** derivative in insect cells.
AUTHOR: Brocks B; Rode H J; Klein M; Gerlach E; Dubel S; Little M; Pfizenmaier K; Moosmayer D
CORPORATE SOURCE: Institute of Cell Biology and Immunology, University of Stuttgart, Germany.
SOURCE: IMMUNOTECHNOLOGY, (1997 Oct) 3 (3) 173-84. Journal code: 9511979. ISSN: 1380-2933.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199712
ENTRY DATE: Entered STN: 19980109
Last Updated on STN: 19980109
Entered Medline: 19971223
AB Single chain antibodies (***scFv***) are usually produced in E. coli, but generation of certain ***scFv*** derivatives, such as complex fusion proteins or glycosylated forms of ***scFv*** is ***restricted*** to eukaryotic expression systems. We investigated the production of soluble mono- and bivalent single chain antibodies (***scFv***) in eukaryotic cells and describe a cassette vector system for mammalian and baculovirus expression which is compatible with an established vector system for bacterial expression and ***phage*** display selection of scFvs. The applied model ***scFv*** was derived from a murine antibody (H398) against human tumor necrosis factor receptor 1 (TNFR60), known to be a potent antagonist of TNF action in its monomeric form and a potential therapeutic agent for treatment of TNF-mediated diseases. Surprisingly, the monomeric ***scFv*** form of H398 (***scFv*** H398) is expressed but not secreted in different mammalian

of the model Ab and its derivatives on antigen binding affinity and neutralisation of TNF activity, we found that the mono- and bivalent form of ***scFv*** H398 possesses the same characteristics as

Furthermore, fusion of the Ig Fc protein to ***scFv*** H398 increase the in vitro half-life at 37 degrees C. We conclude that the described cassette vectors readily allow the eukaryotic expression of mono- and bivalent ***scFv*** derivatives to analyse the influence of valency of ***scFv*** molecules on antigen binding and biological activity.

L19 ANSWER 43 OF 51 MEDLINE DUPLICATE 21
ACCESSION NUMBER: 97184607 MEDLINE
DOCUMENT NUMBER: 97184607 PubMed ID: 9032408
TITLE: Reliable cloning of functional antibody variable domains from hybridomas and spleen cell repertoires employing a reengineered ***phage*** display system.
AUTHOR: Krebber A; Bornhauser S; Burmester J; Honegger A; Willuda J; Bosshard H R; Pluckthun A
CORPORATE SOURCE: Biochemisches Institut der Universitat Zurich, Switzerland.
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (1997 Feb 14) 201 (1) 35-55.
Journal code: 1305440. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X99506; GENBANK-X99507; GENBANK-X99508; GENBANK-X99509
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970321
Last Updated on STN: 19990129
Entered Medline: 19970313

AB A prerequisite for the use of recombinant antibody technologies starting from hybridomas or immune repertoires is the reliable cloning of functional immunoglobulin genes. For this purpose, a standard ***phage*** display system was optimized for robustness, vector stability, tight control of ***scFv*** -delta geneIII expression, primer usage for PCR amplification of variable region genes, ***scFv*** assembly strategy and subsequent directional cloning using a single rare cutting ***restriction*** enzyme. This integrated cloning, screening and selection system allowed us to rapidly obtain antigen binding scFvs derived from spleen-cell repertoires of mice immunized with ampicillin as well as from all hybridoma cell lines tested to date. As representative examples, cloning of monoclonal antibodies against a his tag, leucine zippers, the tumor marker EGP-2 and the insecticide DDT is presented. Several hybridomas whose genes could not be cloned in previous experimental setups, but were successfully obtained with the present system, expressed high amounts of aberrant heavy and light chain mRNAs, which were amplified by PCR and greatly exceeded the amount of binding

scFv [illegible] subsequent to cloning, a compatible vector series to simplify modification, detection, multimerization and rapid purification of recombinant antibody fragments was constructed

L19 ANSWER 44 OF 51 MEDLINE
ACCESSION NUMBER 97299064 MEDLINE
DOCUMENT NUMBER: 97299064 PubMed ID: 9154466
TITLE: Cloning and expression of human V-genes derived from ***phage*** display libraries as fully assembled human anti-TNF alpha monoclonal antibodies.
AUTHOR: Mahler S M; Marquis C P; Brown G; Roberts A; Hoogenboom H R
CORPORATE SOURCE: Department of Biotechnology, University of New South Wales, Kensington, Sydney, Australia.. s.mahler@unsw.edu.au
SOURCE: IMMUNOTECHNOLOGY, (1997 Mar) 3 (1) 31-43.
Journal code: 9511979. ISSN: 1380-2933.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199707
ENTRY DATE: Entered STN: 19970724
Last Updated on STN: 19970724
Entered Medline: 19970717
AB BACKGROUND: With the advent of ***phage*** antibody libraries, access to completely human antibody fragments is feasible, either by direct selection from human antibody libraries, or by guided selection. After selection, Fabs and scFvs may need to be expressed as complete antibodies in mammalian cells for further characterisation, or if effector functions are required. OBJECTIVES: To rebuild and express the human anti-TNF alpha antibody Fab-P3A2 (isolated as a Fab fragment from ***phage*** display libraries by guided selection) as a fully assembled, functional human antibody (gamma-1, lambda) in Sp2/0 myeloma cells, and to perform preliminary characterisation studies of the secreted IgG1 molecule. A further objective was to investigate the kinetics of human antibody production and the stability of antibody secretion in transfectomas cultured in various media formulations. STUDY DESIGN: A tripartite strategy was employed for cloning heavy chain gene (VH)-P3 and light chain gene V lambda-A2-C lambda into mammalian cell expression vectors p alpha Lys-30 and p alpha Lys-17 respectively. The cell line P3A2.B5 was isolated after co-transfection of Sp2 0 mouse myelomas with the constructs, expanded and weaned into a protein free medium. Fully assembled Ig-P3A2 antibody was purified by Protein A affinity chromatography and characterised with respect to size of antibody chains, and affinity for human TNF alpha. Stability of secretion was investigated by extended serial sub-culture and analysis of P3A2.B5 sub-clones. Strategies of media enrichment were tested for any effect on antibody productivity by selected P3A2.B5 sub-clones. RESULTS: The cell line P3A2.B5 secreted an assembled, human antibody Ig-P3A2, with heavy and light chains of molecular weight 55 and 28 KD respectively. Equilibrium capture studies showed Ig-P3A2 to have

[illegible]

unstable with respect to antibody secretion. CONCLUSIONS: We have outlined a method for expression of human V genes as assembled antibodies in Sp2 0 myeloma cells. A cloning strategy for the stable expression of

[illegible]

as assembled human antibodies of the IgGl subclass in Sp2 0 myeloma cells has been described. For maximising specific productivity of antibody-producing cell lines, supplementation of culture media with glucose, glutamine and amino acids increases antibody yield significantly compared to that in conventional media, indicating the latter is stoichiometrically limiting for production purposes.

L19 ANSWER 45 OF 51 MEDLINE DUPLICATE 22
ACCESSION NUMBER: 97225791 MEDLINE
DOCUMENT NUMBER: 97225791 PubMed ID: 9073061
TITLE: An integrated vector system for the eukaryotic expression of antibodies or their fragments after selection from ***phage*** display libraries.
AUTHOR: Persic L; Roberts A; Wilton J, Cattaneo A, Bradbury A, Hoogenboom H R
CORPORATE SOURCE: Societa Italiana per la Ricerca Scientifica, Roma, Italy.
SOURCE: GENE, (1997 Mar 10) 187 (1) 9-18.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970507
Last Updated on STN: 19970507
Entered Medline: 19970429

AB ***Phage*** display is now an established method to select antibody fragments specific for a wide range of diverse antigens. In particular, isolation of human monoclonal antibodies has become a reality and for most purposes bacterial expression of the selected recombinant antibody fragments is sufficient. However, there are some cases where the expression of complete human immunoglobulin in mammalian cells is, if not essential, at least desirable. For this reason we have designed and constructed a set of mammalian expression vectors which permit facile and rapid cloning of antibody genes for both transient and stable expression in mammalian cells. Immunoglobulin genes may be cloned into these expression vectors as V regions or as Fabs for expression as either complete antibodies or as Fab fragments, using ***restriction*** sites which are rare in human V genes. All the important elements in the vectors--promoter, leader sequence, constant domains and selectable markers--are flanked by unique ***restriction*** sites, allowing simple substitution of elements. The vectors have been evaluated using the variable regions from the neutralizing anti-nerve growth factor (NGF) antibody, alphaD11, and the V regions from 2E10, a ***scFv*** selected from a ***scFv*** phagemid library.

DOCUMENT NUMBER: [illegible]
TITLE: A melanoma-specific VH antibody cloned from a fusion ***phage*** library of a vaccinated melanoma patient.
AUTHOR: Cai X; Garen A
CORPORATE SOURCE: [illegible]

Haven, CT 06520-8114, USA.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Jun 25) 93 (13) 6280-5.
Journal code 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U58144
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 19960911
Last Updated on STN: 19980206
Entered Medline: 19960823

AB The human antimelanoma antibody V86 was cloned from a single-chain Fv molecule (***scFv***) fusion ***phage*** library displaying the heavy chain variable domain (VH) and light chain variable domain (VL) repertoire of a melanoma patient immunized with genetically-modified autologous tumor cells. Previous ELISA tests for binding of the V86 fusion ***phage*** to a panel of human metastatic melanoma and carcinoma cell lines and primary cultures of normal melanocytes, endothelial, and fibroblast cells showed that measurable binding occurred only to the melanoma cells. In this communication, the strict specificity of V86 for melanoma cells was confirmed by immunohistochemical staining tests with cultured cells and frozen tissue sections. The V86 fusion ***phage*** stained melanoma cell lines but did not stain carcinoma cell lines or cultured normal cells; V86 also stained specifically the melanoma cells in sections of metastatic tissue but did not stain any of the cells in sections from normal skin, lung, and kidney or from metastatic colon and ovarian carcinomas and a benign nevus. An unexpected finding is that V86 contains a complete VH domain but only a short segment of a VL, domain, which terminates before the CDR1 region. This VL deletion resulted from the occurrence in the VL cDNA of a ***restriction*** site, which was cleaved during construction of the ***scFv*** library. Thus V86 is essentially a VH antibody. The effect of adding a VL domain to V86 was examined by constructing ***scFv*** fusion ***phage*** libraries in which V86 was coupled to Vlambda or Vkappa domains from the original ***scFv*** library of the melanoma patient and then panning the libraries against melanoma cells to enrich for the highest affinity antibody clones. None of the V86-Vlambda clones showed significant binding to melanoma cells in ELISA tests; although binding occurred with most of the V86-Vkappa clones, it was generally weaker than the binding of V86. These results indicate that most of the VL domains in the original ***scFv*** library reduce or eliminate the affinity of V86 for melanoma cells. Accordingly, VH libraries could provide access to anti-tumor antibodies that might not be detected in ***scFv*** or Fab libraries

ACCESSION NUMBER: [illegible]
DOCUMENT NUMBER: 98040658 PubMed ID: 9[illegible]
TITLE: In vitro and in vivo characterisation of a recombinant carboxypeptidase G2-anti-CEA ***scFv*** fusion

AUTHOR: Michael N P; Chester K A; Melton R G; Robson L; Nicholas W; Boden J A; Pedley R B; Begent R H; Sherwood R F; Minton N P
CORPORATE SOURCE: Department of Molecular Microbiology, Centre for Applied Microbiology and Research, Wiltshire, UK.
SOURCE IMMUNOTECHNOLOGY, (1996 Feb) 2 (1) 47-57.
Journal code: 9511979. ISSN: 1380-2933.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH 199712
ENTRY DATE Entered STN: 19980109
Last Updated on STN: 20000303
Entered Medline: 19971215

AB BACKGROUND: There is considerable interest in the specific targeting of therapeutic agents to cancer cells. Of particular promise is a technique known as Antibody-Directed Enzyme Prodrug Therapy (ADEPT). In this approach an enzyme is targeted to the tumour by its conjugation to a tumour specific-antibody tumour. After allowing sufficient time for the conjugate to localise at the tumour and clear from the circulatory system, a relatively non-toxic prodrug is administered. This prodrug is converted to a highly cytotoxic drug by the action of the targeted enzyme localised at the tumour site. OBJECTIVES: To construct gene fusions between the pseudomonad carboxypeptidase G2 (CPG2) gene and DNA encoding MFE-23 (an anti-carcinoembryonic antigen (CEA) single-chain Fv (***scFv***) molecule), derived from a ***phage*** display library. To overexpress the resultant gene fusions in Escherichia coli, and assess the in vitro and in vivo properties of the purified fusion proteins. STUDY DESIGN: To introduce unique cloning ***restriction*** sites into the 5'-end of the CPG2 gene by site-directed mutagenesis to facilitate fusion to the 3'-end of the gene encoding MFE-23 (constructs with or without a flexible (Gly4Ser)3 linker-encoding sequence were designed). To overexpress the resultant gene fusions under transcriptional control of the lac promoter and to direct the fusion proteins produced to the periplasmic space of E. coli through translational coupling to the pelB signal peptide. RESULTS: Biologically active recombinant CPG2::MFE-23 ***scFv*** fusion proteins were produced in E. coli and shown to possess enzyme and anti-CEA activity. Affinity chromatography followed by size exclusion gel filtration yielded approximately 0.7-1.4 mg l from shake flask culture. The fusion protein in which the enzyme and antibody moieties were joined by a linker peptide was shown to be effectively localised in nude mice bearing human colon tumour xenografts, giving favourable tumour to blood ratios. CONCLUSION: MFE-23 ***scFv*** serves as an ideal candidate for the antibody arm of a bacterially expressed fusion protein with CPG2. The biological properties of this recombinant protein suggest that it may be

L17 ANSWER 48 OF 57 MEDLINE DUPLICATE 28
ACCESSION NUMBER: 96095243 MEDLINE
DOCUMENT NUMBER: 96095243 PubMed ID: 8525621

phage display library.
AUTHOR: Ziegler A; Torrance L; Macintosh S M; Cowan G H; Mayo M A
CORPORATE SOURCE: Virology Department, Scottish Crop Research Institute, Invergowrie, Dundee, United Kingdom.
SOURCE: VIROLOGY, (1995 Dec 1) 214 (1) 235-8.
Journal code: 0110674. ISSN: 0042-6822.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199601
ENTRY DATE: Entered STN: 19960219
Last Updated on STN: 19970203
Entered Medline: 19960122
AB Antibody fragments (***scFv***) that bind specifically to particles of cucumber mosaic cucumovirus (CMV) were obtained from a library which encodes a diverse array of synthetic antibody fragments, each displayed on the surface of filamentous bacteriophage. After four rounds of selection and enrichment, several clones were obtained which produced ***scFv*** that bound specifically to purified particles of CMV in ELISA. BstNI digestion of phagemid DNA resulted in the same ***restriction*** pattern for all clones. The nucleotide sequences of three of the clones showed that they belonged to the human VH1 family and that they had a complementarity determining region loop of 7 amino acids. ***Phage*** -displayed antibodies and soluble ***scFv*** secreted by these clones reacted with particles of CMV in sap from infected plants in ELISA. In immunoblotting tests, soluble ***scFv*** preparations reacted with SDS-denatured coat protein extracted from purified preparations of CMV isolates belonging to either subgroup I or II and also with protein extracted by SDS treatment of seeds harvested from naturally infected lupin plants. The results demonstrate the feasibility, and potential applicability, of recombinant antibody methods in plant pathology.

L19 ANSWER 49 OF 51 MEDLINE
ACCESSION NUMBER: 95354944 MEDLINE
DOCUMENT NUMBER: 95354944 PubMed ID: 7628706
TITLE: A single expression system for the display, purification and conjugation of single-chain antibodies.
AUTHOR: Hayashi N; Kipriyanov S; Fuchs P; Welschof M; Dorsam H; Little M
CORPORATE SOURCE: Recombinant Antibody Group, German Cancer Research Center, Heidelberg.
SOURCE: GENE, (1995 Jul 4) 160 (1) 129-30.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands

[illegible]
ENTRY MONTH: 199509
ENTRY DATE: Entered STN: 19950921
Last Updated on STN: 19950921
[illegible]

AB To facilitate the purification and conjugation of single-chain antibodies (***scFv***) selected from a ***phage*** display library, we have incorporated His6, an amber stop codon and a C-terminal Cys into a surface expression vector. The vector also contains a lacIq gene for improving the efficiency of regulation and a sequence coding for a marker peptide.

L19 ANSWER 50 OF 51 MEDLINE DUPLICATE 26
ACCESSION NUMBER: 95309726 MEDLINE
DOCUMENT NUMBER: 95309726 PubMed ID: 7789811
TITLE: A point mutation in a murine immunoglobulin V-region strongly influences the antibody yield in Escherichia coli.
AUTHOR: Duenas M; Ayala M; Vazquez J; Ohlin M; Soderlind E; Borrebaeck C A; Gavilondo J V
CORPORATE SOURCE: Division of Immunotechnology and Diagnostics, Center for Genetic Engineering and Biotechnology, Havana, Cuba.
SOURCE: GENE, (1995 May 26) 158 (1) 61-6.
Journal code: 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article, (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L25854; GENBANK-L25855
ENTRY MONTH: 199507
ENTRY DATE: Entered STN: 19950807
Last Updated on STN: 19950807
Entered Medline: 19950724

AB Recombinant DNA technology has made it possible to produce specific Fab and ***scFv*** antibody (Ab) fragments in prokaryotic host cells. Using vectors designed for periplasmic expression of encoded Ab fragments, we have been studying how the sequence and genetic localization of the light chain (L-chain) variable region gene of a mouse Ab (CB-Nm.1) determined the level of Ab production. The variable region was shown to belong to the V kappa V family and contained a previously unreported Ile72. Nine different Ab constructions were tested in monocistronic (***scFv***) or dicistronic (Fab) operons for their ability to affect the synthesis level of the L-chain. When the gene coding for the L-chain was located downstream from the Fd fragment gene, the substitution of codons encoding Ile by a codon encoding Thr was found to be crucial for any expression of the L-chain fragment. This was, however, not accompanied by an increase in L-chain-specific mRNA, neither was there any change in the size of the mRNA. The fact that the unmutated L-chain protein was produced from cells transformed with certain other constructions indicated that the protein as such was not incompatible with the prokaryotic environment. Together, this suggested that the translation process was involved in the ***restricted*** production of the L-chain. Thus, surprisingly small

L19 ANSWER 51 OF 51 MEDLINE DUPLICATE 27
ACCESSION NUMBER: 93221813 MEDLINE
DOCUMENT NUMBER: 93221813 PubMed ID: 7682084

fragments on filamentous bacteriophages.

AUTHOR: Soderlind E; Lagerkvist A C; Duenas M; Malmborg A C; Ayala M; Danielsson L; Borrebaeck C A

CORPORATE SOURCE: Department of Immunotechnology, Lund University, Sweden.

SOURCE: BIO TECHNOLOGY, (1993 Apr) 11 (4) 503-7.

Journal code: 8309273. ISSN: 0733-222X.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Biotechnology

ENTRY MONTH: 199305

ENTRY DATE: Entered STN: 19950809

Last Updated on STN: 19960129

Entered Medline: 19930507

AB We have used the GroE chaperonins to assist in the packing of a new ***phage*** display vector, pEXmide3. Titers of the packed phagemid increased almost 200-fold from approximately 4 x 10(11) cfu/ml, without coexpression of the GroE proteins, to approximately 7 x 10(13) cfu/ml with their coexpression. Equal titers of non-assisted and assisted phagestocks exhibited the same antigen specificity and ELISA reactivity, indicating the same frequency of displayed Fab-fragments. While the diversity of antibody libraries depends on the bacterial transformation efficiency, the copy number of each antibody is determined by subsequent amplification of the ***phage*** , thus chaperonin assisted phagemid packing in bacteriophage M13 can be used as a general and simple tool to increase the amplification level of expressed Fab fragments. pEXmide3 was developed for display of Fab and single chain Fv-fragments (***scFv***), using ***restriction*** enzymes that do not cut, or cut with low frequencies, in genes encoding immunoglobulin variable domains. The vector allows cloning of genes for the variable domains linking these to predetermined human constant domains or cloning of the entire light and heavy Fab chains. A modification of the pelB leader sequence, with a glutamine to alanine substitution at residue 18, was used for export of the light chain.

d his

(FILE 'HOME' ENTERED AT 13:38:17 ON 05 NOV 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, CAPLUS' ENTERED AT 13:38:42 ON 05 NOV 2002

L1 206501 S MUTAGENESIS

L2 115925 S (LIGHT CHAIN) OR (HEAVY CHAIN)

L3 2100 S L1 AND L2

L4 76274 S BINDING DOMAIN

L9 47 DUP REM L8 (42 DUPLICATES REMOVED)

L10 4583 S BISPECIFIC

L11 242 S L7 AND L10

L13 20 DUP REM L12 (18 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 14:46:42 ON 05 NOV 2002

FILE 'MEDLINE, EMBASE, BIOSIS, CAPLUS' ENTERED AT 14:58:18 ON 05 NOV 2002

L14 40 S RESTRICTED LIGHT CHAIN
L15 15 DUP REM L14 (25 DUPLICATES REMOVED)
L16 123056 S PHAGE
L17 771964 S RESTRIC?
L18 117 S L7 AND L16 AND L17
L19 51 DUP REM L18 (66 DUPLICATES REMOVED)